B16 cont

the death of male F1 ES cells that lack a Y chromosome and are XO F1 ES cells, thereby producing mouse XO F1 ES cells.

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47. (Amended) The method of claim 46, wherein the non-inbred male mouse ES cell clone is an F1 male mouse ES cell.

REMARKS

Information Disclosure Statement

A Second Supplemental Information Disclosure Statement (IDS) was filed on August 27, 2002 and a Fourth Supplemental IDS is being filed concurrently herewith. Copies of the Second Supplemental IDS, Form PTO-1449 and postcard receipt indicating receipt of the Second Supplemental IDS by the PTO on August 27, 2002 are enclosed. Entry and consideration of the Second and Third Supplemental IDSs are respectfully requested.

Rejection of Claims 1, 5, 8, 11, 14, 18, 21 and 41 Under 35 U.S.C. § 112, First Paragraph

The Examiner has maintained the rejection of Claims 1, 5, 8, 11, 14, 18, 21 and 41 under 35 U.S.C. § 112, first paragraph, because, in the Examiner's assessment, the specification lacks "enablement for a method of producing any non-human mammal" by injecting non-inbred ES cells into tetraploid embryos. Paper No. 13, at page 3, lines 14-15. However, the Examiner acknowledges that the specification is enabling for "a method of producing a transgenic mouse or transgenic mutant mouse". Paper No. 13, at page 4, line 22 to page 5, line 1.

Applicants respectfully disagree with the instant rejection for the reasons of record. However, in an effort to advance prosecution in the subject application, Claims 8, 11 and 21 have been cancelled without prejudice. Claims 1, 5, 14, 18 and 41 have been amended to recite a method of producing a mouse or mouse embryo (non-mutant and mutant). Accordingly, Claims 1, 5, 14, 18 and 41 are drawn to subject matter that the Examiner acknowledges to be enabled. Withdrawal of the instant rejection with respect to Claims 1, 5, 14, 18 and 41 is requested.

Rejection of Claim 44 Under 35 U.S.C. § 112, First Paragraph

The Examiner has maintained the rejection of Claim 44 under 35 U.S.C. § 112, first paragraph, because, in the Examiner's assessment, the specification lacks "enablement for a method of producing XO F1 ES cells from mammalian species other than mice". Paper No. 13, at page 9, lines 5-6.

Applicants respectfully disagree with the instant rejection for the reasons of record. However, in an effort to advance prosecution in the subject application, Claim 44 has been amended to recite a method of producing mouse XO F1 ES cells. Accordingly, Claim 44 is drawn to subject matter that the Examiner acknowledges to be enabled. Withdrawal of the instant rejection with respect to Claim 44 is requested.

Provisional Rejection of Claims 1-48 Under 35 U.S.C. § 101 (Double Patenting)

Claims 1-48 have been provisionally rejected under 35 U.S.C. § 101 as claiming the same invention as that of Claims 1-48 of co-pending Application No. 09/957,659.

Claims 1-48 have been canceled in co-pending Application No. 09/957,659, thereby obviating this provisional double patenting rejection.

Rejection of Claims 8-13, 21-26, 31-34, 38 and 39 Under 35 U.S.C. § 112, First Paragraph

Claims 8-13, 21-26, 31-34, 38 and 39 have been rejected under 35 U.S.C. § 112, first paragraph, because, in the Examiner's assessment, the specification "while being enabling for a wild-type or XO mouse . . . does not reasonably provide enablement for any and all mice, mammals or mouse embryos produced according to the methods". Paper No. 13, at page 14, lines 4-10. Applicants respectfully disagree with this assessment.

The standard for enablement under 35 U.S.C. § 112, first paragraph, is whether the claimed invention can be practiced without undue experimentation given the guidance presented in the specification and what was known to the skilled artisan at the time the subject application was filed. The specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art would be able to practice it without undue experimentation. In re Borkowski, 164 U.S.P.Q. 642, 645 (C.C.P.A. 1970). See also M.P.E.P. § 2164.02.

A specification which contains a teaching of how to make and use the full scope of the claimed invention must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. In re Marzocchi, 169 U.S.P.Q. 367, 370 (C.C.P.A. 1971).

Claims 8, 11, 21 and 23 have been cancelled without prejudice. Claims 9, 10, 12, 13, 22, 24-26, 31-34, 38 and 39, as amended, relate to non-mutant and mutant mice and mouse embryos.

The specification teaches methods of producing mice, which can be mutant mice or non-mutant mice. The specification teaches methods of producing mouse embryos, which can be mutant mouse embryos or non-mutant mouse. The specification teaches that these mice and mouse embryos can be produced by tetraploid blastocyst complementation using mouse non-inbred pluripotent cells (see, e.g., page 2, lines 12-15). In particular, the specification teaches that mice can be produced by introducing mouse non-inbred pluripotent cells, such as non-inbred ES cells, into mouse tetraploid blastocysts to produce an embryo and transferring the resulting embryo into an appropriate foster mother, such as a pseudopregnant female (see, e.g., page 6, lines 2-6). The resulting female is maintained under conditions that result in development of live offspring, thereby producing a mouse derived from a single zygote, that which originally gave rise to the non-inbred pluripotent cells (see, e.g., page 6, lines 6-10). The specification teaches that mutant mice can be produced by introducing mouse non-inbred pluripotent cells, such as non-inbred ES cells, comprising at least one mutation or alteration into mouse tetraploid blastocysts to produce an embryo and transferring the resulting embryo into an appropriate foster mother, such as a pseudopregnant female (see, e.g., page 6, lines 12-17). The resulting female is maintained under conditions that result in development of live offspring, thereby producing a mutant mice derived from a single zygote, that which originally gave rise to the non-inbred pluripotent cells (see, e.g., page 6, lines 17-22). The specification teaches that mouse embryos can be produced by introducing mouse non-inbred pluripotent cells, such as non-inbred ES cells, into mouse tetraploid blastocysts and maintaining the resulting tetraploid blastocysts under conditions that result in formation of embryos (see, e.g., page 9, line 28 to page 9, line 2).

Methods for producing tetraploid blastocysts and for introducing non-inbred pluripotent cells into tetraploid blastocysts were also readily available in the art at the time the subject application was filed. Examples of methods for introducing non-inbred pluripotent cells into tetraploid blastocysts are also provided in the specification (see, e.g., page 7, line 29 to page 8, line 1).

Methods for producing mutant mouse non-inbred pluripotent cells that are used to produce mutant mice were readily available in the art at the time the subject application was filed. Examples of methods for producing mutant mouse non-inbred pluripotent cells are also provided in the specification (see, e.g., page 9, lines 10-19).

In the Examples, Applicants provide evidence that non-mutant and mutant mice are produced by tetraploid blastocyst complementation using mouse non-inbred pluripotent cells as described in the specification (see, e.g., page 12, line 6 to page 16, line 10; page 19, Table II). Applicants have thus demonstrated that both non-mutant and mutant mice are produced by tetraploid blastocyst complementation using non-inbred pluripotent cells when following the written disclosure.

Thus, armed with the teachings in the specification and what was known to the skilled artisan at the time the subject application was filed, it would have been a routine matter for one skilled in the art to produce non-mutant and mutant mice and non-mutant and mutant mouse embryos by tetraploid blastocyst complementation using mouse non-inbred pluripotent cells. Accordingly, Applicants submit that the guidance provided in the specification, coupled with what was known to the skilled artisan at the time the subject application was filed, is sufficient to enable the skilled artisan to make and use the full scope of the subject matter of Claims 8-13, 21-26, 31-34, 38 and 39.

The Examiner appears to doubt that the teachings in the specification are sufficient to enable the skilled artisan to practice the full scope of the claimed products without undue experimentation because "the prior art teaches that the phenotype obtained with one species of transgenic animal is not predictive of the same phenotype in another species of transgenic mice". Paper No. 13, at page 15, lines 7-9. The Examiner points to Sigmund (*Arterioscler. Thromb. Vasc. Biol.*, 20:1425-1429 (2000)) and Wall (*Theriogenology*, 45:57-68 (1996)) as providing evidence in support of this position. Applicants respectfully traverse.

Sigmund discusses the source of genetic variability in mutant mouse models, the appropriateness of using inbred mice as controls and strategies to help minimize genetic variation between experimental and control mice. While Sigmund reports that many phenotypes in transgenic and knockout mouse models are influenced by the genetic background in which they are studied and that allelic variation and the interactions between allelic variants influence a particular phenotype, the reference does not conclude that mice and mouse embryos cannot be produced as described in the subject application or provide evidence that would lead one skilled in the art to the conclusion that Applicants' claimed invention is unbelievable. In fact, Sigmund provides strategies to minimize genetic variation and, as such, phenotypic variation, and indicates that a common sense approach provides a framework to identify the causes of phenotypic variation. Accordingly, Sigmund does not provide a sufficient basis to question the enablement provided in the subject specification for the full scope of Claims 9, 10, 12, 13, 22, 24-26, 31-34, 38 and 39, as amended.

Wall identifies several technical limitations that reduce the efficiency of transgenic technology for production of transgenic livestock, including transgene integration, transgene expression and transgene transmission, and provides potential solutions for improving this efficiency. While Wall indicates that "transgene expression and the physiological consequences of transgene products in livestock are *not always* accurately predicted in transgenic mouse studies" (Wall, page 62, lines 7-9; emphasis added), the reference does not conclude that transgenic mouse studies are not correlatable to transgenic livestock. Indeed, Wall states that "a reasonable amount of useful information about transgene function can be derived from transgenic mouse studies" (Wall, page 62, third paragraph). The reference, in proposing strategies to improve efficiency of transgene expression, integration and transmission in livestock would not lead one skilled in the art to the conclusion that Applicants' claimed invention is unbelievable. Accordingly, Wall does not provide a sufficient basis to question the enablement provided in the subject specification for the full scope of Claims 9, 10, 12, 13, 22, 24-26, 31-34, 38 and 39, as amended.

The Examiner also appears to doubt that the teachings in the specification are sufficient to enable the skilled artisan to practice the full scope of the claimed products without undue experimentation because "[w]ithin mice the phenotype arising from insertion or deletion of even

a well-characterized gene is equally unpredictable". Paper No. 13, at page 15, lines 19-20. Citing Doetschman (*Lab. Animal Sci.*, 49:137-143 (1999)), the Examiner alleges that "the phenotype arising from any given mutation or genetic manipulation of a transgenic mouse is highly unpredictable and in some cases requires empirical experimentation to uncover." Paper No. 13, at page 16, lines 7-9. Applicants respectfully traverse.

Doetschman reviewed the literature focusing on questions of unexpected phenotypes, apparent lack of phenotype, gene redundancy and effect of genetic background on phenotypic variation on knockout mice. He provided possible reasons for unexpected knockout phenotypes and strategies for interpreting unexpected knockout phenotypes and for dealing with apparent lack of knockout phenotypes. Based on a review of the literature, Doetschman found that there is little gene redundancy in mammals, that knockout phenotypes exist even if none are immediately apparent and that by investigating phenotypes in colonies of mixed genetic background, more phenotypes may be revealed, a better understanding of the molecular or cellular mechanisms underlying the phenotype may result and modifier gene(s) may be discovered. Accordingly, Doetschman does not support the Examiner's conclusion that a phenotype arising from any given mutation or genetic manipulation of a transgenic mouse is highly unpredictable. The reference does not provide evidence that would lead one skilled in the art the conclusion that Applicants' claimed invention is unbelievable. Accordingly, Doetschman does not provide a sufficient basis to question the enablement provided in the subject specification for the full scope of Claims 9, 10, 12, 13, 22, 24-26, 31-34, 38 and 39, as amended.

Furthermore, the need for empirical experimentation to determine a phenotype is not a sufficient basis to question the enablement provided in the specification, particularly since such experimentation is routine in the art.

The Examiner alleges that "the specification is silent regarding how to use any and all non-human mammals beyond a general statement that they can be used to screen drugs". Paper No. 13, at page 16, lines 13-14. The claims have been amended to recite that the non-human mammals are mice, thereby rendering this aspect of the rejection moot.

The Examiner contends that "in order to use any given animal the skilled artisan must have a means to measure the phenotype of that particular animal and changes in that phenotype" and that the "means to measure any given phenotype is unique to that phenotype and there does

not exist a general means to assess a change in phenotype." Paper No. 13, at page 16, lines 18-20. Applicants respectfully traverse.

It was within the skill of the skilled artisan at the time the subject application was filed to determine the phenotype of a mouse produced using the methods described in Applicants' specification and to determine changes in phenotype relative to a normal (wildtype) mouse. For example, the skilled artisan can determine a phenotype and changes in phenotype by observing the mouse and comparing it to a normal mouse. In this case, the skilled artisan can physically observe the mouse, as well as perform behavioral, histological, cytological and other analysis. Phenotype can also be determined by crossing mice carrying a particular mutation to produce offspring homozygous for the mutation. Additionally, the location of a mutation can be mapped by breeding homozygous mice with a wildtype strain. Methods to determine and measure a phenotype were well known and described in the art at the time the subject application was filed. See, e.g., Crawley, J., *What's Wrong With My Mouse? Behavioral Phenotyping of Transgenic and Knock-Out Mice*, Wiley-Liss, NY (2000); Hogan, B. *et al.* (Eds.), *Manipulating the Mouse Embryo: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, Plainville, NY (1994); Wassarman, P.M. *et al.* (Eds.), *Methods In Enzymology: Guide To Techniques In Mouse Development*, Academic Press, San Diego, CA (1993); Jackson, I.J. *et al.*, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press, Oxford, England (2000).

The skilled artisan can also determine genotype using a variety of methods that were well known and described in the art at the time the subject application was filed. See, e.g., Hogan, B. *et al.* (Eds.), *Manipulating the Mouse Embryo: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, Plainville, NY (1994); Cox, R.D. *et al.*, "Genome Mapping and Claining of Mutation Using Yeast Artificial Chromosome", *in* Wassarman, P.M. *et al.* (Eds.), *Methods In Enzymology: Guide To Techniques In Mouse Development*, Academic Press, San Diego, CA pages 637-653 (1993); Jackson, I.J. *et al.*, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press, Oxford, England (2000). For example, genotype can be determined by extracting tissue from a mouse, analyzing the DNA from the extracted tissue for vector or plasmid DNA used to introduce DNA into ES cells.

The Examiner also alleges that "production of a useful phenotype through expression or knockout of a given gene in all species of mammal, or expression or knock out of any and all genes in the mouse is beyond the capabilities of the ordinary skilled artisan." Paper No. 13, at page 17, lines 1-4. Applicants respectfully disagree with the Examiner's assessments, which are unsupported by evidence.

It was within the skill of the skilled artisan at the time the subject application was filed to target and mutate or overexpress specific genes. As discussed above, methods for producing mutant non-inbred pluripotent ES cells that are used to produce mutant mice as described in accordance with Applicants' specification were readily available in the art at the time the subject application was filed.

The specification teaches that the mutant mouse model serves as a model of a condition that occurs in a different mammalian species (see, e.g., page 3, lines 25-28). Such a condition includes neurological, muscular or respiratory conditions, cancer, viral infection, arthritis (see, e.g., page 3, lines 25-27), and conditions caused by or associated with a genetic alteration (see, e.g., page 4, lines 4-5). Such conditions, along with defining characteristics (symptoms, signs, pathology, phenotypes, etc.) were well known and described in the art at the time the subject application was filed. See, e.g., Beers *et al.* (Eds.), *The Merck Manual of Diagnosis and Therapy*, 17th edition, Merck Research Laboratories (1999) (for human conditions). Mouse models of conditions that occur in different mammalian species were known and described in the art at the time the subject application was filed. Mouse models can also be designed using information and methods that were readily available in the art at the time the subject application was filed. See, e.g., Popko (Ed.), *Mouse Models in the Study of Genetic Neurological Disorders (Advances in Neurochemistry, V. 9)*, 1st edition, Kluwer Academic Publishers (1999); Tarui *et al.* (Eds.), *Insulitis and Type I Diabetes: Lessons from the Nod Mouse*, Academic Press (1997); Mohr *et al.* (Eds.), *Cancer Pathology of Tumours in Laboratory Animals: Tumours of the Mouse (Iarc Scientific Publications, No. 111)*, 2nd edition, Oxford University Press (1994); and Sundberg (Ed.), *Handbook of Mouse Mutations with Skin and Hair Abnormalities: Animal Models and Biomedical Tools*, CRC Press (1994). The specification is not required to disclose what is well known in the relevant art at the time the subject application was filed. See, e.g., Lindemann Maschinenfabrik G.m.b.H. v. American Hoist & Derrick Co., 221 U.S.P.Q. 481, 489

(Fed. Cir. 1984); Hybritech, Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and In re Wands, 8 U.S.P.Q.2d 1400, 1402 (Fed. Cir. 1988).

Reconsideration and withdrawal of this rejection of Claims 9, 10, 12, 13, 22, 24-26, 31-34, 38 and 39, as amended, under 35 U.S.C. § 112, first paragraph, are respectfully requested.

Rejection of Claim 40 Under 35 U.S.C. § 112, First Paragraph

The Examiner has maintained the rejection of Claim 40 under 35 U.S.C. § 112, first paragraph, because, in the Examiner's assessment, the claim contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and use the invention. However, the Examiner acknowledges that the specification is enabling for "any mouse established to be a phenotypic model of a mammalian disease". Paper No. 13, at page 17, lines 18-20.

Claim 40 is dependent from Claim 14, which has been amended to recite a method of producing mutant mice. As discussed above, methods for producing mutant non-inbred pluripotent ES cells that are used to produce mutant mice were readily available in the art at the time the subject application was filed. Examples of methods for producing mutant non-inbred pluripotent ES cells are also provided in the specification (see, e.g., page 9, lines 10-19).

As discussed above, it was within the skill of the skilled artisan at the time the subject application was filed to determine the phenotype of a mouse produced using the methods described in Applicants' specification and to determine changes in phenotype relative to a normal (wildtype) mouse. For example, the skilled artisan can determine a phenotype and changes in phenotype by observing the mouse and comparing it to a normal mouse. In this case, the skilled artisan can physically observe the mouse, as well as perform behavioral, histological, cytological and other analysis. Phenotype can also be determined by crossing mice carrying a particular mutation to produce offspring homozygous for the mutation. Additionally, the location of a mutation can be mapped by breeding homozygous mice with a wildtype strain. Methods to determine and measure a phenotype were well known and described in the art at the time the subject application was filed. See, e.g., Crawley, J., *What's Wrong With My Mouse? Behavioral Phenotyping of Transgenic and Knock-Out Mice*, Wiley-Liss, NY (2000); Hogan, B. *et al.* (Eds.),

Manipulating the Mouse Embryo: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, Plainville, NY (1994); Wassarman, P.M. *et al.* (Eds.), *Methods In Enzymology: Guide To Techniques In Mouse Development*, Academic Press, San Diego, CA (1993); Jackson, I.J. *et al.*, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press, Oxford, England (2000).

The skilled artisan can also determine genotype using a variety of methods that were well known and described in the art at the time the subject application was filed. See, e.g., Hogan, B. *et al.* (Eds.), *Manipulating the Mouse Embryo: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, Plainville, NY (1994); Cox, R.D. *et al.*, "Genome Mapping and Claining of Mutation Using Yeast Artificial Chromosome", *in* Wassarman, P.M. *et al.* (Eds.), *Methods In Enzymology: Guide To Techniques In Mouse Development*, Academic Press, San Diego, CA pages 637-653 (1993); Jackson, I.J. *et al.*, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press, Oxford, England (2000). For example, genotype can be determined by extracting tissue from a mouse, analyzing the DNA from the extracted tissue for vector or plasmid DNA used to introduce DNA into ES cells.

Accordingly, armed with Applicants' teachings in the specification, it would be a routine matter to identify a candidate drug having a therapeutic or preventive effect on a condition that occurs in a mammal by using a mutant mouse that is a model of the condition to screen drugs for the ability to treat or prevent the condition, wherein the mutant mouse is produced by tetraploid blastocyst complementation using mouse non-inbred pluripotent cells as described in the specification. Reconsideration and withdrawal of this rejection of Claim 40 under 35 U.S.C. § 112, first paragraph, are respectfully requested.

<u>Rejection of Claims 1, 4, 8, 9, 15, 21, 22 and 41 Under 35 U.S.C. § 112, First Paragraph</u>

Claims 1, 4, 8, 9, 15, 21, 22 and 41 have been rejected under 35 U.S.C. § 112, first paragraph, because, in the Examiner's assessment, the specification "does not reasonably provide enablement for a method of producing any animal from any pluripotent cell other than an ES cell or for any animal produced from any pluripotent cell other than an ES cell." Paper No. 13, at page 19, lines 2-4. However, the Examiner acknowledges that the specification is enabling for

"method of producing a mouse wherein ES cells are introduced into tetraploid blastocysts and a mouse produced by the method". Paper No. 13, at page 18, lines 20-21.

Applicants respectfully disagree with the instant rejection for the reasons. However, in an effort to advance prosecution in the subject application, Claims 8, 15 and 21 have been cancelled without prejudice. Independent Claims 1 and 41 have been amended to recite a method of producing a mouse (non-mutant and mutant) and to recite that the pluripotent cells are mouse non-inbred pluripotent ES cells. Accordingly, independent Claims 1 and 41 and dependent Claims 4, 9 and 22 are drawn to subject matter that the Examiner acknowledges to be enabled. Withdrawal of the instant rejection with respect to Claims 1, 4, 9, 22 and 41 is requested.

<u>Rejection of Claims 19, 20, 25, 26 and 47 Under 35 U.S.C. § 112, Second Paragraph</u>

Claims 19, 20, 25, 26 and 47 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

In particular, Claim 19 has been rejected as indefinite in the recitation of the phrase "the mutant non-human mammal is a mouse" because no antecedent basis is provided for this phrase in Claim 18, from which Claim 19 depends. Claims 20, 25 and 26 have also been rejected indefinite because they depend from Claim 19.

Claim 19 has been cancelled without prejudice, thereby obviating this aspect of the rejection under 35 U.S.C. § 112, second paragraph.

Claim 47 has been rejected as indefinite in the recitation of the phrase "the non-inbred male ES cell" because Claim 46, from which Claim 47 depends, is limited to "non-inbred mouse ES cells".

Claim 47 has been amended to recite "the non-inbred male mouse ES cell clone" in place of "the non-inbred male ES cell", thereby obviating this aspect of the rejection under 35 U.S.C. § 112, second paragraph.

Rejection of Claims 1-3, 8-12, 14-16 and 21-23 Under 35 U.S.C. § 102(b)

Claims 1-3, 8-12, 14-16 and 21-23 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Wang *et al.* (*Mechanisms of Development*, 62:137-145 (1997)).

Applicants respectfully disagree with the Examiner's conclusion that Claims 1-3, 8-12, 14-16 and 21-23 are anticipated by the teachings of Wang *et al.* The Court of Appeals for the Federal Circuit has stated that "[u]nder 35 U.S.C. § 102, anticipation requires that each and every element of the claimed invention be disclosed in a prior art reference." Akzo N.V. v. International Trade Comm., 11 U.S.P.Q.2d 1241, 1245 (Fed. Cir. 1986) (citations omitted).

Claims 2, 3, 8, 11, 15, 16, 21 and 23 have been cancelled without prejudice. Claims 1 and 14, as amended, relate to methods of producing mice (non-mutant and mutant) by introducing mouse non-inbred pluripotent ES cells into mouse tetraploid blastocysts by injection. Claims 9, 10 and 22, as amended, relate to mice (non-mutant and mutant) produced by tetraploid complementation using non-inbred pluripotent ES cells. Claims 12 and 13 relate to mouse embryos produced by tetraploid complementation using non-inbred ES cells.

In contrast, Wang *et al.* teach a method of producing mice from embryonic stem cells by introducing inbred ES cells into tetraploid mouse blastocysts. Wang *et al.* do not teach or suggest the use of non-inbred pluripotent ES cells, in the generation of mutant and non-mutant mice or mouse embryos. Accordingly, the methods disclosed by Wang *et al.* are different from the methods of Claims 1 and 14, as amended. As such, Claims 1, 9, 10, 12-14 and 22, as amended, are not anticipated by the Wang *et al.* reference.

Reconsideration and withdrawal of this rejection of Claim 1, 9, 10, 12-14 and 22 under 35 U.S.C. § 102(b) are requested.

Rejection of Claims 1, 2, 8, 9, 14, 15, 21, 22, 27, 31, 33, 35, 38 and 41 Under 35 U.S.C. § 102(b)

Claims 1, 2, 8, 9, 14, 15, 21, 22, 27, 31, 33, 35, 38 and 41 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Ueda *et al.* (*Exp. Anim.*, 44:205-210 (1995)) as evidenced by Yagi *et al.* (*Anal. Biochem.*, 214:70-76 (1993)).

Applicants respectfully disagree with the Examiner's conclusion that Claims 1, 2, 8, 9, 14, 15, 21, 22, 27, 31, 33, 35, 38 and 41 are anticipated by the teachings of Ueda *et al.* Anticipation

requires that each and every element of the claimed invention be disclosed in a prior art reference.

Claims 2, 8, 15 and 21 have been cancelled without prejudice. Claims 1,14, 27 and 35, as amended, relate to methods of producing mice (non-mutant and mutant) by introducing mouse non-inbred pluripotent ES cells into mouse tetraploid blastocysts by injection. Claims 9 and 22, as amended, relate to mice (non-mutant and mutant) produced by tetraploid complementation using non-inbred pluripotent ES cells. Claims 31, 33 and 38 relate to mouse embryos produced by tetraploid complementation using non-inbred ES cells.

In contrast, Ueda *et al.* teach a method of producing mice from TT2 ES cells by aggregation with tetraploid morulae. Ueda *et al.* do not teach or suggest producing mutant and non-mutant mice and mouse embryos by injection of non-inbred pluriopotent ES cells into tetraploid blastocysts. Accordingly, the methods disclosed by Ueda *et al.* are different from the methods of Claims 1, 14, 27 and 35, as amended. As such, Claims 1, 9, 14, 22, 27, 31, 33, 35 and 38 are not anticipated by the Ueda *et al.* reference.

Reconsideration and withdrawal of this rejection of Claims 1, 9, 14, 22, 27, 31, 33, 35, 38 and 41 under 35 U.S.C. § 102(b) are requested.

Rejection of Claims 18, 19, 25, 27, 28, 35, 36 and 45 Under 35 U.S.C. § 103(a)

Claims 18, 19, 25, 27, 28, 35, 36 and 45 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Uchida *et al.* (*Animal Science Tech.*, 66:361-367 (1995)) as evidenced by Yagi *et al.* (*Anal. Biochem.*, 214:70-76 (1993)) in view of either Ueda *et al.* (*Exp. Anim.*, 44:205-210 (1995)) or Wang *et al.* (*Mechanisms of Development*, 62:137-145 (1997)).

Teachings of the Cited References

Uchida *et al.*

Uchida *et al.* is cited by the Examiner as teaching "the means to make XO F1 ES cells capable of producing a viable mouse and a line of such cells were known in the art at the time of filing of the instant Application". Paper No. 13, at page 28, lines 10-12. In particular, the Examiner states that Uchida *et al.* teach "producing a non-human mammalian embryo comprising

injecting non-human (i.e. mouse) non-inbred (i.e. TT2) ES cells into non-human blastocysts and maintaining the resulting blastocysts under conditions that result in formation of embryos." Paper No. 13, at page 27, lines 14-17. The Examiner also states that Uchida *et al.* teach "the method wherein the ES cells used are XO F1 ES cells." Paper No. 13, at page 27, line 18. Applicants respectfully disagree with the Examiner's assessments of the cited reference.

Uchida *et al.* teach a method of identifying XO ES cells within a mixed population of mouse XY ES cells. Importantly, Uchida *et al.* teach the use of their isolated XO ES cells in producing female germ-line chimeras and embryos. Uchida *et al.* do not teach or suggest the generation of mice and mouse embryos using tetraploid blastocyst injection and without the need to first create chimeric intermediates.

<u>Ueda *et al.*</u>

Ueda *et al.* is cited by the Examiner as teaching "a method of making a mouse entirely derived from cultured ES cells by introducing ES cells into a tetraploid blastocyst was also known in the art at the time the instant application was filed." Paper No. 13, at page 28, lines 12-14. In particular, the Examiner states that Ueda *et al.* teach "production of mice derived entirely from mouse TT2 ES cells by (a) introducing TT2 ES cells into tetraploid mouse blastocysts under conditions that result in production of an embryo; and (b) transferring the resulting embryo into a foster mother which is maintained under conditions that result in development of live offspring". Paper No. 13, at page 27, line 21 to page 28, line 4.

Importantly, Ueda *et al.* teach a method of producing mice from TT2 ES cells by aggregation with tetraploid morulae. Ueda *et al.* report that their approach "cannot be considered as feasible to routinely achieve germline transmission from genetically manipulated ES cells" (Ueda *et al.*, page 210, column 1, lines 9-11). As such, Ueda *et al.* teach away from a method of producing mice and mouse embryos by introducing non-inbred ES cells into tetraploid blastocysts. In particular, Ueda *et al.* do not teach or suggest producing mutant and non-mutant mice and mouse embryos by injection of non-inbred pluriopotent ES cells into tetraploid blastocysts.

<u>Wang *et al.*</u>

Wang *et al.* is cited by the Examiner as teaching "a method of making a mouse entirely derived from cultured ES cells by introducing ES cells into a tetraploid blastocyst was also known in the art at the time the instant application was filed." Paper No. 13, at page 28, lines 12-14. In particular, the Examiner states that Wang *et al.* teach "production of mice entirely from embryonic stem cells by introducing ES cells into tetraploid mouse blastocysts under conditions that result in production of an embryo and transferring the resulting embryo into a foster mother which is maintained under conditions that result in development of live offspring". Paper No. 13, at page 28, lines 5-9.

Importantly, Wang *et al.* teach a method of producing mice from embryonic stem cells by introducing inbred ES cells into tetraploid mouse blastocysts. Wang *et al.* do not teach or suggest the use of non-inbred pluripotent ES cells, in the generation of mutant and non-mutant mice or mouse embryos.

Combination of References

In support of the rejection, the Examiner alleges that:

> It would have been obvious to one of ordinary skill in the art to combine the teachings of Uchida *et al.* and Ueda *et al.* or Wang *et al.* according to the methods of the instant application to produce the claimed XO female mouse, as the teachings provide the means to make XO F1 ES cells from the TT2 cell line, a line of XO F1 ES cells derived from TT2 cells that is capable of generating viable mice, and the means to produce a mouse entirely derived from TT2 or other ES cells.
>
> Motivation to combine these teachings comes from Wang *et al.* who teaches in the first paragraph of the second column on page 143, "the practical benefit of [production of mice from ES cells using tetraploid blastocysts] should be emphasized as it may save time and money for generating mutant mouse strains from cultured ES cells and allows a rapid access to mutant fetuses and mice, a potential advantage for many investigators in the field of mouse genetics".
>
> In the absence of evidence to the contrary, the skilled artisan would also have a reasonable expectation of success in combining the teachings of Uchida *et al.* with the teachings of Ueda *et al.* or Wang *et al.* This is particularly true in the case of

> Ueda *et al.* who demonstrate that the parental TT2 cell line from which the XO F1 ES cells of Uchida *et al.* is derived can be used in their method of making a mouse.

Paper No. 13, at page 28, line 14 to page 29, line 7.

Applicants respectfully disagree that Claims 18, 19, 25, 27, 28, 35, 36 and 45, as amended, are obvious in view of the cited combinations of references.

A *prima facie* case of obviousness is established only if the teachings of the cited art would have suggested the claimed invention to one of ordinary skill in the art with a reasonable expectation of successfully achieving the claimed results. In re Vaeck, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). Both the suggestion and the reasonable expectation of success must be found in the prior art, not Applicants' disclosure. Id.

The Court of Appeals for the Federal Circuit has stated that "[t]he proper approach to the obviousness issue must start with the claimed invention *as a whole*." See, e.g., Kimberley-Clark Corp. v. Johnson & Johnson Co., 223 U.S.P.Q. 603, 609 (Fed. Cir. 1984). See also Lindemann Maschinenfabrik G.m.b.H. v. American Hoist & Derrick Co., 221 U.S.P.Q. 481, 488 (Fed. Cir. 1984). It is not proper to pick and choose among individual elements of assorted prior art references to recreate the claimed invention. Smithkline Diagnostics Inc. v. Helena Laboratories Corp., 8 U.S.P.Q.2d 1468, 1475 (Fed. Cir. 1988); Akzo N.V. v. International Trade Comm., 11 U.S.P.Q.2d 1241, 1246 (Fed. Cir. 1986).

Claim 19, 28 and 36 have been cancelled without prejudice. Claim 18, as amended, relates to a method of producing a mutant mouse embryo by tetraploid blastocyst complementation using mutant mouse non-inbred ES cells. Claim 27, as amended, relates to a method of producing a mutant mouse by tetraploid blastocyst complementation using mutant mouse non-inbred ES cells. Claim 35, as amended, relates to a method of producing a mouse by tetraploid blastocyst complementation using mouse non-inbred ES cells. Claim 25 relates to a mutant mouse embryo produced by tetraploid complementation using mutant mouse non-inbred ES cells. Claim 45 relates to XO female mice produced by tetraploid complementation using non-inbred ES cells, specifically XO F1 ES cells.

None of the cited references (Uchida *et al.*, Wang *et al.*, Ueda *et al.*), alone or in combination, would have suggested the claimed invention to one of ordinary skill in the art at the

time the invention was made with a reasonable expectation of success. More specifically, one of ordinary skill in the art would not have been able to predict with a reasonable expectation of success, given the combination of cited references, whether mutant mouse embryos would be produced by tetraploid blastocyst complementation using mouse non-inbred pluripotent ES cells. One of ordinary skill in the art would not have been able to predict with a reasonable expectation of success, given the combination of cited references, whether a mouse (non-mutant and mutant) would be produced by tetraploid blastocyst complementation using mouse non-inbred ES cells. One of ordinary skill in the art would not have been able to predict with a reasonable expectation of success, given the combination of cited references, whether an XO female mice would be produced by tetraploid complementation using X1 F1 ES cells.

As discussed above, Uchida *et al.* teach a method of identifying inbred XO ES cells within a mixed population of mouse XY ES cells and the use of the isolated inbred XO ES cells in producing female germ-line chimeras and embryos. Wang *et al.* teach the generation of ES-derived mice and embryos by tetraploid blastocyst injection, but do not teach or suggest using non-inbred pluripotent ES cells. Ueda *et al.* teach a method of producing mice from TT2 ES cells by aggregation with tetraploid morulae and report that their approach "cannot be considered as feasible to routinely achieve germline transmission from genetically manipulated ES cells" (Ueda *et al.*, page 210, column 1, lines 9-11). As such, Ueda *et al.* teach away from a method of producing mice and mouse embryos by introducing non-inbred ES cells into tetraploid blastocysts. In particular, Ueda *et al.* do not teach or suggest producing mutant and non-mutant mice and mouse embryos by injection of non-inbred pluriopotent ES cells into tetraploid blastocysts. Accordingly, neither the Wang *et al.* reference nor the Ueda *et al.* reference cures the deficiencies of the Uchida *et al.* reference. Thus, the cited references, either alone or in combination, would not have suggested the claimed invention to one of ordinary skill in the art, at the time the invention was made, with a reasonable expectation of success. At best, the cited references merely indicate that specific isolated elements and/or features recited in the claims are known. This is insufficient to render the claimed invention *prima facie* obvious.

As discussed above, even assuming, *arguendo*, that a *prima facie* case of obviousness exists, which it does not, a patent applicant can rebut a *prima facie* case of obviousness by a showing of "unexpected results", e.g., by showing that the claimed invention exhibits some

superior property or advantage that a person of ordinary skill in the art would have found surprising or unexpected. See, e.g., In re Soni, 34 U.S.P.Q.2d 1684, 1687 (Fed. Cir. 1995).

As discussed in Amendment A, filed September 27, 2002, Applicants obtained the unexpected and surprising result that 60 pups were produced after injection of 344 tetraploid blastocysts with 6 different ***non-inbred*** ES cell lines (i.e., 6 different F1 ES cell lines) and that 51 (85%) survived to adulthood (see, e.g., page 15, lines 18-20; and page 19, Table 2). In contrast, 20 pups were produced after injection of 312 tetraploid blastocysts with 4 different ***inbred*** ES cell lines and only 1 survived to adulthood (see, e.g., page 15, lines 13-18; and page 18, Table 1). The magnitude and efficiency of these results using non-inbred ES cells (i.e., the number of pups that were produced (60) and the number of pups that survived to adulthood (51)), relative to the number of pups that were produced using inbred ES cells (20) and that survived to adulthood (1), could not have been predicted from the cited references.

Moreover, Applicants demonstrated the unexpected and surprising result that live, adult mice, entirely derived from ES cells can be generated from F1 ES cells even after long-term passage of the cells in culture or after consecutive rounds of drug selection (see, e.g., page 16, lines 7-10). In particular, Applicants found that no impairment of the resulting ES cell-tetraploid pups was noted after either 15 or 25 passages (see, e.g., page 15, lines 27-28) or after 2 consecutive rounds of drug selection (see, e.g, page 16, lines 1-7). The magnitude of these results could not have been predicted from the cited references, particularly since, as reported in the specification, it has previously been shown that continued passage of ES cells is detrimental to their developmental potency (see, e.g., page 15, lines 22-24).

Additionally, Applicants' unexpected and surprising results have been recognized by those skilled in the art (see Cross, J.C., *Proc. Natl. Acad. Sci. USA*, 98(11):5949-5951 (2001); attached hereto as the Exhibit).

Accordingly, even assuming, *arguendo*, that a *prima facie* case of obviousness exists, which it does not, it has been overcome by Applicants' unexpected results.

Applicants' claimed invention also satisfies a long-felt need which was recognized, persistent and not solved by others. In the past two decades, considerable effort has been invested in developing a method for producing mutant and transgenic mice at a higher efficiency that can be used routinely. In order to produce desired mutant mice efficiently using methods

that were available prior to Applicants' invention, a skilled artisan would generally first produce chimeras and breed the chimeras to produce homozygous offspring. Production of offspring which are not chimeric generally entails two breeding cycles for each gene. This is the case for each mutation to be introduced and, if offspring exhibiting more than one mutation are desired, additional breeding cycles are needed. For example, if mutant mice bearing six different alterations (e.g., six different genes) are to be produced, approximate breeding time will be two years. Producing desired genetically manipulated mice requires considerable time and resources using methods that were available prior to Applicants' invention. With Applicants' invention, mice can be generated without the need to first create a chimeric intermediate. The ability to derive offspring directly from ES cells in accordance with Applicants' claimed invention without the need to produce chimeric intermediates is a distinct advantage because it avoids the time-consuming and expensive step of producing chimera and facilitates the generation of offspring with multiple genetic alterations at a higher efficiency. The generation of mice in accordance with Applicants' invention provides a simple routine procedure for directly deriving mice with complex genetic alterations without the need to create a chimeric intermediate. The tetraploid technology in combination with the use of non-inbred ES cells allows assembly or production of multiple genetic alterations in the same ES cell clone by consecutive gene targeting cycles *in vitro*. The resulting multiply targeted non-inbred ES cell clone is introduced into tetraploid blastocysts to produce an embryo that is then transferred to an appropriate foster mother and permitted to develop to term. Thus, a transgenic mouse with one or multiple desired or selected genetic alterations can be generated without the need for production of chimeric founders and outbreeding with wildtype mice. Accordingly, Applicants' invention satisfies a long-felt unfulfilled need in the art that was not solved by others.

Reconsideration and withdrawal of the rejection of Claims 18, 25, 27, 35 and 45 under 35 U.S.C. § 103(a) are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If

the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Helen Lee

Helen Lee
Registration No. 39,270
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
Dated: June 16, 2003

MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A method of producing a mouse [non-human mammal, referred to as an ES non-human mammal], wherein mouse non-inbred pluripotent ES cells are introduced into mouse tetraploid blastocysts by injection [of the same mammalian species] under conditions that result in production of an embryo and the resulting embryo is transferred into a foster mother which is maintained under conditions that result in development of live offspring, wherein said foster mother is a mouse [the pluripotent cells are non-inbred pluripotent cells].

4. (Amended) The method of claim 1 [3], wherein injection is piezo microinjection.

5. (Amended) A method of producing a [non-human mammalian] mouse embryo comprising injecting [non-human] mouse non-inbred ES cells into [non-human] mouse tetraploid blastocysts and maintaining the resulting tetraploid blastocysts under conditions that result in formation of embryos, thereby producing a [non-human mammalian] mouse embryo.

7. (Twice Amended) The method of claim 5 [6], wherein the mouse non-inbred ES cells are mutant mouse non-inbred ES cells and are injected into non-human tetraploid blastocysts by piezo microinjection.

9. (Amended) A mouse produced by the method of claim 1 [2].

10. (Amended) A mouse produced by the method of claim 4 [3].

12. (Amended) A mouse embryo produced by the method of claim 5 [6].

14. (Amended) A method of producing a mutant mouse [non-human mammal], wherein mouse non-inbred pluripotent ES cells comprising at least one mutation in genomic DNA are

introduced into mouse tetraploid blastocysts by injection [of the same mammalian species] under conditions that result in production of an embryo and the resulting embryo is transferred into a foster mother which is maintained under conditions that result in development of live offspring, thereby producing a mutant mouse [non-human mammal], wherein said foster mother is a mouse [the pluripotent cells are non-inbred pluripotent cells].

17. (Amended) The method of claim 14 [16], wherein injection is piezo microinjection.

18. (Amended) A method of producing a mutant mouse [non-human mammalian] embryo comprising injecting mutant mouse [non-human] non-inbred ES cells into [non-human] mouse tetraploid blastocysts and maintaining the resulting tetraploid blastocysts under conditions that result in formation of embryos, thereby producing a mutant mouse [non-human mammalian] embryo.

20. (Amended) The method of claim 18 [19], wherein mutant mouse non-inbred ES cells are injected into [non-human] mouse tetraploid blastocysts by piezo microinjection.

22. (Amended) A mutant mouse produced by the method of claim 14 [15].

24. (Amended) A mutant mouse [embryo] produced by the method of claim 17.

25. (Amended) A mutant mouse embryo produced by the method of claim 18 [19].

27. (Amended) A method of producing a mutant mouse, comprising: (a) introducing mouse non-inbred ES cells comprising at least one mutation in genomic DNA into mouse tetraploid blastocysts by injection, thereby producing mouse blastocysts containing mouse non-inbred ES cells; (b) maintaining the product of (a) under conditions that result in production of embryos; (c) introducing an embryo into a pseudopregnant female mouse; [:] and (d) maintaining the female mouse into which the embryo is introduced under conditions that result in development of live offspring, thereby producing a mutant mouse.

29. (Amended) The method of claim 27 [28], wherein [microinjection] injection is piezo microinjection.

35. (Amended) A method of producing a mouse, comprising: (a) introducing mouse non-inbred ES cells into mouse tetraploid blastocysts by injection, thereby producing mouse blastocysts containing mouse non-inbred ES cells; (b) maintaining the product of (a) under conditions that result in production of embryos; (c) introducing an embryo into a pseudopregnant female mouse; [:] and (d) maintaining the female mouse into which the embryo is introduced under conditions that result in development of live offspring, thereby producing a mouse.

37. (Amended) The method of claim 35 [36], wherein injection [microinjection] is piezo microinjection.

41. (Twice Amended) A method of producing a mutant mouse [non-human mammal], wherein mouse non-inbred pluripotent ES cells comprising at least one mutation in genomic DNA are introduced into mouse tetraploid blastocysts by injection [of the same mammalian species] under conditions that result in production of an embryo and the resulting embryo is transferred into a foster mother which is maintained under conditions that result in development of live offspring, wherein said foster mother is a mouse [the pluripotent cells are non-inbred pluripotent cells].

44. (Twice Amended) A method of producing [mammalian] mouse XO F1 ES cells, comprising introducing into [mammalian] mouse male F1 ES cells a negative selection marker, under conditions appropriate for insertion of the negative selection marker in the Y chromosome of [mammalian] mouse male F1 ES cells, thereby producing a mixture of [mammalian] mouse male F1 ES cells comprising male F1 ES cells in which the negative selection marker in inserted in the Y chromosome and other male F1 ES cells, some of which do not contain a Y chromosome; subjecting the resulting mixture to conditions that result in the death of male F1 ES cells in which the Y chromosome has the negative selection marker inserted therein

and do not result in the death of male F1 ES cells that lack a Y chromosome and are XO F1 ES cells, thereby producing [mammalian] <u>mouse</u> XO F1 ES cells.

47. (Amended) The method of claim 46, wherein the non-inbred male <u>mouse</u> ES cell <u>clone</u> is an F1 male mouse ES cell.